Age-dependent association of thyroid function with brain morphology and microstructure

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ABSTRACT

Background Thyroid hormone (TH) is crucial during neurodevelopment but high levels of TH have been linked to neurodegenerative disorders. No data on the association of thyroid function with brain imaging in the general population are available.

Methods We therefore investigated the association of thyroid-stimulating hormone and free thyroxine (FT4) with MRI-derived total intracranial volume, brain tissue volumes and diffusion tensor imaging (DTI) measures of white matter microstructure in 4,683 dementia- and stroke-free participants (mean age 60.2, range 45.6-89.9 years).

Results Higher FT4 levels were associated with larger total intracranial volumes (β=6.73mL, 95% confidence interval 2.94-9.80). Higher FT4 levels were also associated with larger total brain and white matter volumes in younger individuals, but with smaller total brain and white matter volume in older individuals (p-interaction 0.02). There was a similar interaction by age for the association of FT4 with mean diffusivity on DTI (p-interaction 0.026).

Conclusions These results are in line with differential effects of TH during neurodevelopmental and neurodegenerative processes and can improve understanding of the role of thyroid function in neurodegenerative disorders.

INTRODUCTION

Thyroid hormone impacts different essential neuronal processes including neurogenesis, myelination, and neural differentiation in childhood and throughout adulthood.[1,2] Already during intrauterine neurodevelopment, thyroid hormone impacts on several processes including neuronal cell proliferation, differentiation and migration.[1,3] Suboptimal thyroid hormone availability during early life can have profound impact on brain function later in life. This is illustrated by the link between congenital hypothyroidism and cretinism, a condition characterized by severely impaired physical and mental development. Mild forms of both low and high thyroid function during the gestational period have been associated with a lower child IQ and differences in brain morphology during later life.[4]

However, the effects of thyroid hormone on the brain are age dependent because in addition to its effects during development, thyroid hormone is also related to neurodegeneration. In older adults, higher thyroid function has been associated with a higher risk of neurodegenerative disorders and poorer cognition.[5,6] A meta-analysis of cohort studies showed that higher thyroid function is associated with higher risk of cognitive impairment.[7] We previously described an increased risk of dementia with high-normal to high thyroid function and a protective effect of low and low-normal thyroid function.[8] This risk did not seem to be explained by cardiovascular risk factors or subclinical vascular brain damage. The underlying mechanisms explaining the link between thyroid function and dementia are yet unclear, but possible and yet unexplored pathways are through subclinical changes in microstructural organization or brain tissue atrophy.

Owing to the link with cognitive impairment, we hypothesized that thyroid hormone could have adverse effects on processes affecting brain volumes and white matter microstructure in older age. We also hypothesized that this effect could be age-dependent, due to the different effects of thyroid hormone during the neurodevelopmental period. Therefore, we investigated the association of thyroid function with intracranial brain volume (as a marker of development), total brain, white matter and grey matter volumes on MRI (as markers of neurodegeneration). Furthermore, we tested whether the association of thyroid function and diffusion tensor imaging (DTI) measures related to white matter microstructural organization were different in younger versus older participants.

METHODS

Setting

The study was performed in the context of the Rotterdam Study (RS), a prospective population-based cohort study that investigates determinants and occurrence of cardiovascular, neurological, ophthalmologic, psychiatric, and endocrine diseases in the middle-aged and elderly population. The aims and design of the Rotterdam Study have been described in detail elsewhere.[9] We included participants from three independent cohorts within the Rotterdam Study. The RS Cohort 1 (RSI) includes participants aged 55 years and older and baseline data were collected during 1990-1993. RS Cohort II (RSII) includes participants aged 55 years and older and baseline data were collected from 2000-2001. For the RS Cohort 3 (RSIII), persons included were aged 45 years and over and baseline data were collected from 2006 to 2008. Thyroid function assessment was determined in a random subset of 9,689 participants in all three cohorts and brain MRI was included in the core protocol of the Rotterdam Study since 2005. The study protocol was approved by the Medical Ethics Committee of the Erasmus University and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)". All included participants provided a written informed consent in accordance with the Declaration of Helsinki to participate in the study and to obtain information from their family physicians.

Study population

We included all participants from the Rotterdam Study, cohort I wave 3, cohort II wave I and cohort III wave I, with thyroid function measurements, MRI measurements and free of dementia at baseline (n=5104). We excluded 248 participants with prevalent stroke and with MRI-defined cortical infarcts and 173 participants using thyroid function altering medication (levothyroxine, anti-thyroid drugs, amiodarone or corticosteroids). Final study population included 4,683 participants of which 3,852 also had DTI measurements.

Assessment of thyroid function

Thyroid function was measured through thyroid-stimulating hormone (TSH) and free thyroxine (FT4) using the same methods and assay for all cohorts (The electrochemiluminescence immunoassay for thyroxine and thyrotropine, "ECLIA", Roche) in serum samples stored at -80°C. We determined the reference values for normal range TSH as 0.4-4.0 mIU/L and FT4 as 11-25 pmol/L (= 0.85-1.95 ng/dL) according to national guidelines as well as our previous studies.[8,10]

MRI acquisition and analysis

Multi-sequence brain MR Imaging was performed on a 1.5 tesla MRI scanner (GE Signa Excite). The imaging protocol and sequence details were described extensively elsewhere.[11] Scans were automatically segmented supra tentorially into grey matter, white matter, cerebrospinal fluid (CSF) and background tissue. Intracranial volume (ICV) (excluding the cerebellum and surrounding CSF) was estimated by summing total grey and white matter and CSF volumes.[12] Total brain volume was estimated by summing total grey and white matter volumes.[12] A post-processing white matter lesion classification, based on the FLAIR image and the tissue segmentation, was used to obtain white matter lesion volumes (natural-log transformed to account for their skewed distribution).[13] All segmentations were visually inspected and were corrected if needed. Cortical infarcts were visually rated on structural sequences, and were classified as cortical infarcts in case of involvement of cortical grey matter. In a subset of our study population (N=2,449) cerebellar volume was processed automatically using FreeSurfer 4.5. This procedure, based on probabilistic information obtained from a manually labeled training set, assigns a neuroanatomical label to each voxel in an MRI volume. This is explained in more detail elsewhere.[14] In this subset we computed intracranial volume, including cerebellar volume, and total cerebellar volume.

Diffusion-MRI processing

For characterization of white matter microstructural organization with DTI, a single shot, diffusion-weighted spin echo echo-planar imaging sequence was performed. Maximum b-value was 1000 s/mm^2 in 25 non-collinear directions and three volumes were acquired without diffusion weighting (b-value = 0 s/mm^2). For the diffusion acquisition, due to technical issues 1165 participants were scanned with the phase and frequency encoding directions swapped leading to a mild ghost artifact.[15] We corrected for this potential confounder in our analyses. Diffusion data were pre-processed using a standardized pipeline (including correction for motion and eddy currents) as previously described.[16] A diffusion tensor model was estimated in each voxel, and co-registration between structural imaging and diffusion image space was performed.[15] Through averaging of the diffusion measurements inside the normal appearing white matter (voxels with white matter lesions were excluded from the analysis) we obtained global mean fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity, and axial diffusivity.[15] The median time between thyroid function measurement and MRI scan was 0.21 years (interquartile range: 0.06-10.16).

Assessment of other variables

Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer after 5 minutes of rest with the participants in sitting position. The mean

of two consecutive measurements was used. Information regarding the use of blood pressure lowering medication for the indication of hypertension was derived from structured home interviews and linkage to pharmacy records. Serum total and high density lipoprotein (HDL) cholesterol were measured in fasting serum by enzymatic method. Smoking information was derived from baseline questionnaires and categorized in never, previous and current smokers. Alcohol consumption was documented as intake in grams per day. Body-mass index (BMI) was calculated as weight kilograms divided by height in meters squared. History of diabetes mellitus was defined by a repeated (two measurements within one year) impaired fasting glucose ≥ 7 mmol/L or a non-fasting glucose of ≥ 11 mmol/L (when fasting samples were absent) or use of anti-glycemic medication at baseline.[17] Educational level was assessed during a baseline home interview and people were classified into 7 categories: from low level of education (primary only) to high (university). Prevalent dementia and clinical stroke were ascertained as previously described.[18,19] In short, participants were evaluated for dementia using a three strep protocol. All participants were screened using the Mini-Mental State Examination (MMSE)[20] and Geriatric Mental State schedule (GMS).[21] Persons scoring ≤ 25 on the MMSE or >0 on the GMS underwent an examination and informant interview with the Cambridge Examination for Mental Disorders of the Elderly. Persons suspicious of having dementia underwent further neuropsychological testing if necessary. Furthermore, in addition to the above screening method, persons were continuously monitored for the dementia diagnosis through computerized linkage of the study database and medical records of the general practitioner's office and Regional Institute for Outpatient Mental Health Care (RIAGG). The accepted DSM-III-R criteria were used for the dementia diagnosis[22].

Statistical analysis

We used ordinary least squares linear regression models with restricted cubic splines at three knots,[23] which provided the best fit with the data without overfitting, for all analyses, to account for possible non-linear associations. Primary analyses for brain volume measurements were adjusted for age, sex, cohort, time between laboratory measurement and MRI scan and intracranial volume. We included intracranial volume as a covariate in our model to correct for the inter-individual variability in head size.[24] In a second model, we additionally adjusted for several cardiovascular risk factors including total cholesterol, HDL-cholesterol, systolic blood pressure, diastolic blood

pressure, smoking, prevalent diabetes mellitus, BMI, alcohol use and educational level. For the analyses of intracranial volume, we did not adjust for the variable itself. Primary analyses for diffusion measurements adjusted for age, sex, cohort, time between laboratory measurement and MRI scan, intracranial volume, white matter volume and white matter lesion volume. A second model additionally adjusted for total cholesterol, HDL-cholesterol, systolic blood pressure, diastolic blood pressure, smoking, prevalent diabetes, BMI, alcohol use and educational level. TSH was natural log-transformed for all analyses to approximate normality and interaction of TSH or FT4 with age (as a continuous measure) or sex was tested for all analyses. We conducted sensitivity analyses 1) constricting analyses to the reference range of thyroid function (n=4141) and 2) excluding participants with time interval above 1 year between laboratory measurement and MRI scan (n=2527). In order to quantify the effects in different age groups we additionally stratified our main analyses by the mean age of our population (~ 60 years of age). Missing covariates (< 5% for all covariates but alcohol [6.7%]) were imputed using multiple imputations creating 5 data sets according to the Markov Chain Monte Carlo method and pooled subsequently (IBM SPSS Statistics for Windows, version 21.0. Armonk, NY). In our multiple comparisons correction we took the main analyses (FT4, TSH with the 9 different outcomes (intracranial volume, total brain volume, total white matter volume, total grey matter volume, cerebellar volume, FA, MD, radial diffusivity, and axial diffusivity) into account. We estimated the number of independent tests by using the variance of the eigenvalues of the correlation matrix of the 11 variables used in our main analyses. We calculated the number of independent tests using the following formula: M_{eff}= 1 +(M-1)(1-var (λ_{obs})/M) with M is number of tests, M_{eff} is the number of independent tests.[25,26] Based on this formula M_{eff} was 5.668331474. Afterwards, using the Šidák formula: α sidak = 1 - ((1 - α)^(1/M_{eff})) our corrected significance level was p< 0.0081.[25] Statistical analyses were conducted and plots were produced using R statistical software (rms, Himsc, visreg packages, R-project, Institute for Statistics and Mathematics, R Core Team (2013), Vienna, Austria, version 3.0.2).

RESULTS

We included a total of 4683 participants, with an average age of 60.2 years (range 45.6-89.9) of which 54.9% were female (**Table 1**). As the results of the two tested models are comparable we present both for illustration, but only report the most adjusted model in the manuscript. Linearity assumption was met for all main analyses and indicated in tables if otherwise.

Table 1 Characteristics of the 4,683 study participants

Variable	Mean (SD) [a]
Age, years	60.2 (7.3)
Female sex n, %	2,571 (54.9)
TSH, ImU/L median (IQR)	1.97 (1.36 - 2.78)
FT4, pmol/L	15.5 (2.1)
Intracranial volume, mL	1140.0 (115.5)
Grey matter volume, mL	529.4 (53.6)
White matter volume, mL	409.2 (59.1)
White matter lesion volume, mL, median, IQR	2.9 (1.7-6.0)
Mean fractional anisotropy	0.34 (0.02)
Mean diffusivity, 10^{-3} mm^2/s	0.74 (0.03)
BMI, kg/m^2	27.1 (4.0)
Total cholesterol, mmol/L	5.70 (1.02)
HDL-cholesterol, mmol/L	1.42 (0.41)
Systolic blood pressure, mmHg	134.8 (19.3)
Diastolic blood pressure, mmHg	79.9 (10.9)
Prevalent diabetes n, %	399 (8.5)
Smoking	
Current n, %	1,029 (22.0)
Past n, %	2,211 (47.2)
Never n, %	1,443 (30.8)
Alcohol use, grams per day, median, IQR	15.0 (6.3-21.4)
Time between thyroid function measurement and scan (in years) median, IQR	0.21 (0.06-10.16)

[a] Values are means and (standard deviation) unless otherwise specified.
There were 13 participants with missing values for BMI, 5 total cholesterol, 17 HDL-cholesterol, 14 systolic and diastolic, 17 smoking, 18 for prevalent diabetes and 313 for alcohol use.
Abbreviations: BMI = body-mass index; TSH = thyroid-stimulating hormone, FT4 = free thyroxine; SD = Standard deviation; IQR = inter-quartile range

Brain tissue volumes and intracranial volume

Higher FT4 levels were associated with larger intracranial volume with a beta (β) of 6.23 mL per one unit increase of FT4 pmol/L (95% Confidence Interval [CI], 2.80, 9.66, **Table 2**), and the association was not different according to age (**Figure 1**, **Supplemental Table 1**, **Supplemental Table 2**). There was no association of TSH levels with intracranial volume. Higher FT4 levels were associated with larger brain volume overall (β 2.26, CI, 1.10, 3.43, **Table 2**) and white matter volume in particular (β 1.43, CI, 0.25, 2.62, **Table 2**), but not in elderly (**Figure 1**, **Supplemental Table 2**). The p for interaction of age with total brain volume was 0.002 and for age with white matter volume was 0.038 (**Supplemental Table 1**). The associations of FT4 and TSH with total brain volume were mainly attributable to white matter and not gray matter volume (**Table 2**). Higher levels of TSH were associated with smaller brain volumes (β -1.35, CI, -2.26,

-0.45, **Table 2**). Higher FT4 levels were associated with a larger white matter volume (β 1.43, CI, 0.24, 2.62), but not gray matter volume (β 0.78, CI, -0.25, 1.80). Higher TSH levels were associated with a smaller white matter volume (β - 1.58, CI,-2.50, - 0.67), but not grey matter volume (β 0.19, CI, -0.61, 0.99). Constricting analyses to the reference range of thyroid function or excluding participants with time interval between laboratory measurement and MRI scan > 1 year did not change effect estimates (**Table 3**). There was no significant interaction of FT4 or TSH with sex on the association with any of the studied outcomes (p> 0.10). There was no significant interaction of TSH with age on the association with any of the studied outcomes (p> 0.35, **Supplemental Table 1**).

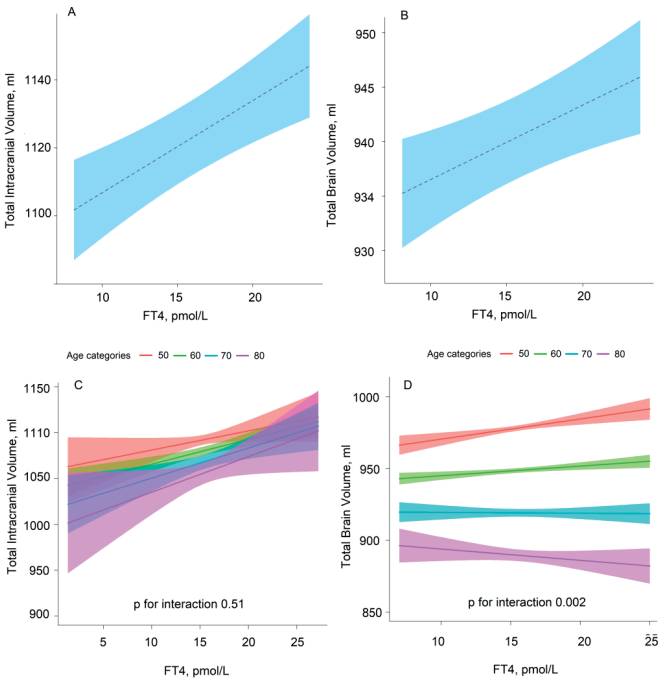

Figure 1. Total intracranial and brain volume according to FT4 serum values
Plot A) depicts the association of FT4with intracranial volume and plot B) depicts the association of FT4 with total brain volume. Plot C) depicts the interaction of FT4 with age continuously for the intracranial volume analysis and D) depicts interaction of FT4 with age continuously for the total brain volume analysis. All analyses are adjusted for age, sex, cohort and time between laboratory measurements and MRI scan, and the analyses with brain volume were additionally adjusted for intracranial volume.

Total cerebellar volume

The additional analysis using a subgroup of 2249 persons with cerebellar volume data measured with FreeSurfer, yielded a similar albeit a more pronounced association of FT4 with intracranial volume (β 10.13, CI, 3.61, 16.64, **Supplemental Table 3**). FT4 was positively associated with cerebellar volume (β 0.68.13, CI, 3.61, 16.64, **Supple-**

mental table 3). No associations were observed for TSH with intracranial volume or cerebellar volume (**Supplemental Table 3**).

Table 2 Association of TSH or FT4 with intracranial, total, white and grey matter brain volume measurements

Variable	Total intracranial volume β (95% CI)	Total brain volume β (95% CI)	Total white matter volume β (95% CI)	Total grey matter volume β (95% CI)
TSH				
Model 1	0.39 (-2.32, 3.09)	-1.37 (-2.28, -0.46)	-1.58 (-2.50, -0.67)	0.21 (-0.59, 1.01)
Model 2	0.64 (-2.04, 3.32)	-1.37 (-2.27, -0.47)	-1.55 (-2.47, -0.63)	0.18 (-0.61, 0.98)
FT4				
Model 1	7.23 (3.78, 10.67)	1.95 (0.78, 3.12)*	1.43 (0.25, 2.61)	0.47 (-0.55, 1.49)
Model 2	6.23 (2.80, 9.66)	2.26 (1.10, 3.43)	1.43 (0.25, 2.62)	0.78 (-0.24, 1.81)

Model 1: age, sex, cohort, time between laboratory measurement and MRI scan and intracranial volume Model 2= Model 1 + total cholesterol, HDL-cholesterol, systolic blood pressure, diastolic blood pressure, smoking, prevalent diabetes, BMI, alcohol use and educational level. * p for non-linearity 0.054; Abbreviations: CI = confidence interval; FT4 = free thyroxine; MRI= Magnetic resonance imaging; OR = odds ratio; TSH = thyroid stimulating hormone

Table 3 Sensitivity analyses of association of TSH or FT4 with MRI intracranial and brain volume measurements

Variable		Total intracranial volume β (95% CI)	Total brain volume β (95% CI)	Total white matter volume β (95% CI)	Total grey matter volume β (95% CI)
Reference range (n= 4,141)*					
TSH	Model 1	-0.91 (-5.40, 3.58)	-1.40 (-2.89, 0.09)	-1.88 (-3.40, -0.35)	0.48 (-0.84, 1.81)
	Model 2	-0.87 (-5.34, 3.59)	-1.30 (-2.78, 0.17)	-1.76 (-3.28, -0.22)	0.45 (-0.87, 1.78)
FT4	Model 1	8.31 (4.25, 12.36)	2.32 (0.87, 3.76)**	1.56 (0.07, 3.04)	0.60 (-0.60, 1.80)
	Model 2	7.17 (3.12, 11.22)	2.53 (1.09, 3.97)**	1.41 (-0.08, 2.90)	0.99 (-0.22, 2.20)
Excluding participants with time interval between laboratory measurement and MRI scan > 1 years (n= 2,527)					
TSH	Model 1	1.42 (-2.7, 5.56)	-1.41 (-2.74, -0.08)	-1.22 (-2.55, 0.11)	-0.24 (-1.42, 0.49)
	Model 2	1.54 (-2.57, 5.65)	-1.50 (-2.82, -0.17)	-1.23 (-2.57, 0.11)	-0.30 (-1.48, 0.88)
FT4	Model 1	6.78 (1.95, 11.61)	2.57 (1.01, 4.13)**	2.36 (0.79, 3.93)	0.08 (-1.29, 1.46)
	Model 2	5.40 (0.57, 10.22)	2.95 (1.39, 4.52)**	2.42 (0.83, 4.00)	0.43 (-0.96, 1.82)

Model 1: age, sex, cohort, time between laboratory measurement and MRI scan and intracranial volume Model 2= Model 1 + total cholesterol, HDL-cholesterol, systolic blood pressure, diastolic blood pressure, blood pressure lowering medication with indication of hypertension, smoking, prevalent diabetes, BMI, alcohol use and educational level.

* Reference ranges for TSH were 0.4-4.0 mIU/L and for FT4 were 11-25 pmol/L. ** non-linearity of association $p < 0.05$. Abbreviations: CI = confidence interval; FT4 = free thyroxine; MRI= Magnetic resonance imaging; OR = odds ratio; TSH = thyroid stimulating hormone

White matter microstructural organization

There was no overall association of TSH or FT4 with diffusion properties of white matter, neither FA nor MD (**Table 4**). However, there was a significant interaction with age for the association of FT4 and MD (p for interaction 0.026, **Figure 2**, **Supplemental Table 1**). In older participants, higher FT4 values were associated with a lower FA (albeit not statistically significant interaction, p = 0.052, **Supplemental Table 1**) and higher MD, generally indicating reduced matter microstructural integrity (**Supplemental Table 4**). In contrast, in younger participants, higher FT4 levels were associated with a higher FA and lower MD, generally indicating increased white matter integrity. The associations for radial and axial diffusivity followed the same pattern as mean diffusivity (**Figure 2**). All results survive the threshold for significance after multiple comparisons correction except for the association of FT4 in the full range with white matter volume.

Table 4 Association of full range TSH or FT4 with Z-scores of Diffusion Tensor Imaging parameters of white matter (n=3,852)

	FA β (95% CI)	MD β (95% CI)	RD β (95% CI)	AD β (95% CI)
TSH				
Model 1	-0.00(-0.03, 0.03)	0.01(-0.01, 0.03)	0.01(-0.01, 0.03)	0.01(-0.01, 0.03)
Model 2	-0.00(-0.03, 0.03)	0.01(-0.01, 0.03)	0.01(-0.01, 0.03)	0.01(-0.01, 0.03)
FT4				
Model 1	-0.01(-0.05, 0.02)	0.02(-0.01, 0.04)	0.01(-0.01, 0.04)	0.02(-0.01, 0.04)
Model 2	-0.01(-0.04, 0.03)	0.01(-0.02, 0.04)	0.01(-0.02, 0.04)	0.01(-0.01, 0.04)

Model 1: age, sex, cohort, time between laboratory measurement and MRI scan, intracranial volume, white matter volume and white matter lesions. Model 2= Model 1 + total cholesterol, HDL-cholesterol, systolic blood pressure, diastolic blood pressure, blood pressure lowering medication with indication of hypertension, smoking, prevalent diabetes, BMI, alcohol use and educational level. *Abbreviations: CI = confidence interval; FA = fractional anisotropy; FT4 = free thyroxine; MD = mean diffusivity; RD = radial diffusivity; AD = axial diffusivityTSH = thyroid stimulating hormone*

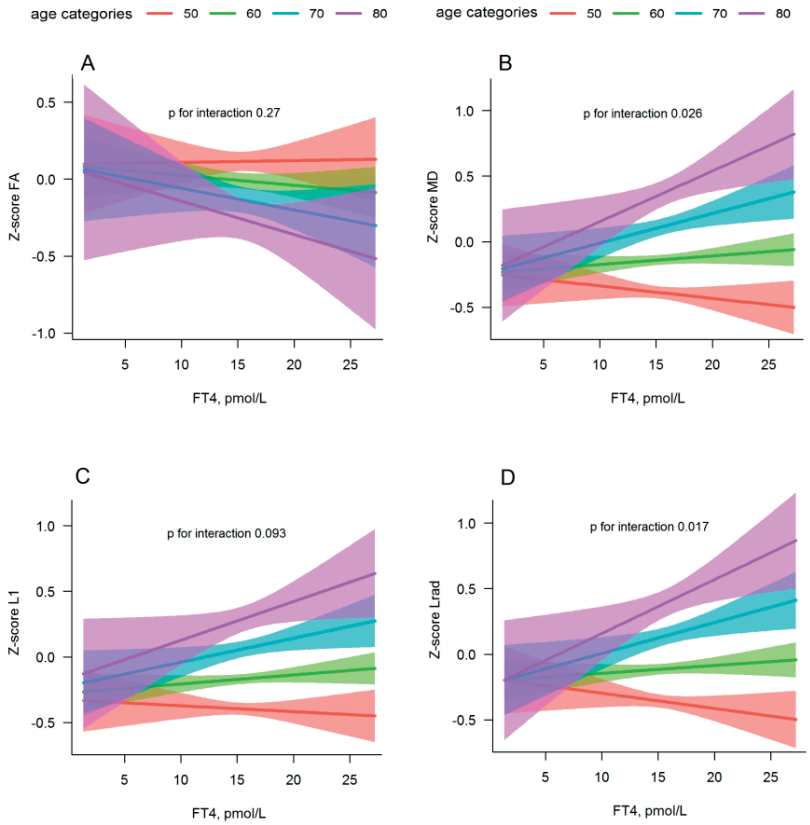

Figure 2. DTI measurements according to FT4 serum values

Plot depicting the interaction of FT4 with age for z-scores of A) fractional anisotropy, B) mean diffusivity, C) radial diffusivity and D) axial diffusivity . All analyses are adjusted for age, sex, cohort, time between laboratory measurements and MRI scan, intracranial volume, white matter and white matter lesion volume.

DISCUSSION

We report an association of higher FT4 with larger intracranial volume, independent of age. Higher FT4 levels are also associated with a larger total brain volume, mainly attributable to white matter volume. However this association was age-dependent. In older participants (over ~70 years old) higher FT4 levels were associated with a smaller total brain volume, primarily white matter. We also found a differential effect of age on the association of FT4 with DTI measures that can be linked to white matter integrity. In older participants, higher FT4 were associated with DTI measures reflecting poorer white matter integrity. In younger participants (especially those <50 years), higher FT4 levels were associated with DTI measures reflecting better white matter integrity, primarily lower MD. These findings could indicate an age-dependent effect of thyroid hormone on brain morphology and microstructural organization, beneficial in younger

age and deleterious in older age. To our knowledge, there are no previous studies assessing the association of thyroid function with brain volumes and white matter microstructure in the general population. Based on our results, we hypothesize that the findings in younger participants could reflect a positive role of thyroid hormone balance in myelination, sustainability and protection of neurons during early stages of life. In contrast, in older age, high thyroid function could be deleterious by causing neuronal damage and in turn neurodegeneration.

Thyroid hormone is important for growth, development and metabolism in virtually all organs and effects on the brain are numerous. Fetal neurogenesis is thyroid hormone dependent and both lack and excess of thyroid hormone availability can hamper brain development and has deleterious effects on brain morphology.[1,2,27] In older age, mainly high thyroid function has been linked to neurodegeneration and cognitive impairment.[6,7,10] Higher thyroid hormone levels are related to a higher basal metabolic rate and oxygen consumption. In turn, this can affect oxidative stress, either due to increased reactive oxygen species production or lower activity of antioxidants, potentially leading to oxidative damage.[28,29] These effects are reported to be tissue specific, but oxidative damage may be most pronounced in metabolic active organs such as the brain possibly leading to negative effects on neuronal integrity.[29] Free radical injury has been suggested to associate with white matter changes on DTI.[30] With microstructural changes thought to accumulate to macrostructural tissue change, this could be one of the pathways explaining the association of thyroid function with lower white matter volume and poorer integrity in elderly, potentially also the previously described relation with the risk of dementia. The pathophysiology of dementia is multifactorial, but implication of oxidative stress has also been proposed.[31,32] Alternatively, a common genetic predisposition could underlie the association of thyroid function with dementia and white matter integrity.

Thyroid function is known to affect several cerebrovascular risk factors. However, deleterious cardiovascular risk factors, such as dyslipidemia and increased blood pressure, are mainly consequences observed in hypothyroidism. Furthermore, we previously described lack of association of thyroid function with small vessel disease on MRI, including white matter lesions, lacunar infarcts and cerebral microbleeds.[8] Also, adjusting for various cardiovascular risk factors in the current study did not change the associations meaningfully. This, together with the current results, suggests that the association of thyroid function with measures of white matter microstructure in elderly is independent of these risk factors and mediated through other pathways (e.g. oxidative stress). Thyroid hormone also has distinct effects on myelin formation and regeneration.[33,34] However, thyroid hormone and thyroid hormone repletion is mainly associated with acceleration of myelination and remyelination in different animal studies and patient populations.[33-35] This demonstrates the potential complexity of the

pathophysiology[2] and more research is needed to unravel the exact pathophysiological link between thyroid function, white matter microstructure and dementia. Discovery of underlying pathways is not only needed to understand the pathophysiology of dementia, but also to identify persons at risk and determine promising treatment targets. Another implication of our study may lie in the possible effects of overtreatment of hypothyroidism. In recent years, physicians have commenced levothyroxine treatment in people at lower serum TSH thresholds, i.e. milder cases of hypothyroidism.[36] It was found that in patients treated with levothyroxine for hypothyroidism, a substantial proportion was not within treatment target, with over 10% being overtreated and actually classifying as iatrogenic hyperthyroidism.[36] Our study results suggest that endogenous high FT4 values may negatively affect brain volume and brain tissue in older age. Although we have not investigated whether high thyroid function due to levothyroxine (i.e. exogenous thyroxine) use has comparable effects on brain volumes and DTI measurements as endogenous thyroid hormone, we speculate that this is plausible. Further research is needed to confirm this hypothesis.

Strengths of our study include the large sample size and availability of detailed phenotypical information. Also, we were able to adjust for a wide variety of confounders. Nevertheless, residual confounding cannot be excluded in an observational study, even with adjustments for the large number of potential confounders performed in our analyses. Another limitation of our study is that thyroid function was measured only once, which is a limitation for most observational cohort studies, and therefore changes over time could not be assessed. A sensitivity analysis limited to participants with thyroid function within the reference range (n=4141), which are known to be relatively stable over time,[37,38] yielded similar results. Nevertheless, our results need to be confirmed in study preferably with a longitudinal design. Furthermore; no conclusions can be drawn on the causality of the associations due to the cross-sectional design. We used averaged diffusion parameters, aggregated over all the normal-appearing white matter voxels. This did not allow us to assess brain changes on a more regional level. Finally, the Rotterdam Study constitutes of mainly Caucasian participants of 45 years and older, so our results may be less generalizable to younger or other ethnic populations.

Conclusions

In summary, our study shows that higher FT4 levels are associated with larger brain volumes and higher white matter microstructural integrity in younger individuals, but not in elderly. Furthermore, our results highlight the need for caution in overtreatment in mild hypothyroidism. Thyroid hormone excess is a risk factor for dementia and further studies should evaluate whether this is indeed mediated through poorer white matter microstructural integrity.

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